

What is claimed is:

1. A method for detecting *Alicyclobacillus* and *Geobacillus* in a test sample, the method comprising
 - (a) providing an oligonucleotide set comprising:
 - (i) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 1327 through nucleotide position 1460 shown in Figure 1;
 - (ii) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 1327 through nucleotide position 1460 shown in Figure 1, said second consecutive sequence being downstream from said first consecutive sequence,; and
 - (iii) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is which is identical to a third consecutive sequence within the sequence of nucleotide position 1327 through nucleotide position 1460 shown in Figure 1, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences;
 - (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and
 - (c) determining the presence of PCR products of step (b), wherein the presence of a PCR product in the sample is indicative of contamination of the test sample by *Alicyclobacillus* or *Geobacillus* or both.
2. The method of claim 1 wherein the forward primer has a sequence selected from the group consisting of SEQ ID NO 1, SEQ ID NO 5, SEQ ID NO 9, SEQ ID NO 13, SEQ ID NO 17, and SEQ ID NO 20.
3. The method of claim 1 wherein the reverse primer has a sequence selected from the group consisting of SEQ ID NO 4, SEQ ID NO 8, SEQ ID NO 12, SEQ ID NO 16, SEQ ID NO 19, and SEQ ID NO 23.

4. The method of claim 1 wherein the probe has a sequence selected from the group consisting of SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 18, SEQ ID NO 21, and SEQ ID NO 22.

5. A method for detecting *Alicyclobacillus* and *Geobacillus* in a test sample, the method comprising

(a) providing a oligonucleotide set comprising:

(i) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 334 through nucleotide position 485 shown in Figure 5;

(ii) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 334 through nucleotide position 485 shown in Figure 5, said second consecutive sequence being downstream from said first consecutive sequence; and

(ii) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is which is identical to a third consecutive sequence within the sequence of nucleotide position 334 through nucleotide position 485 shown in Figure 5, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences;

(b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and

(c) determining the presence of PCR products of step (b), wherein the presence of a PCR product in the sample is indicative of contamination of the test sample by *Alicyclobacillus* or *Geobacillus* or both.

6. The method of claim 5 wherein the forward primer has a sequence selected from the group consisting of SEQ ID NO 28, and SEQ ID NO 29.

7. The method of claim 5 wherein the reverse primer has a sequence selected from the group consisting of SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 36, and SEQ ID NO 37.

8. The method of claim 5 wherein the probe has a sequence selected from the group consisting of SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, and SEQ ID NO 33.

9. A method for detecting *Alicyclobacillus* and *Geobacillus* in a test sample, the method comprising

(a) providing a oligonucleotide set comprising:

(i) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 752 through nucleotide position 813 shown in Figure 1;

(ii) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 752 through nucleotide position 813 shown in Figure 1, said second consecutive sequence being downstream from said first consecutive sequence; and

(ii) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is which is identical to a third consecutive sequence within the sequence of nucleotide position 752 through nucleotide position 813 shown in Figure 1, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences;

(b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and

(c) determining the presence of PCR products of step (b), wherein the presence of a PCR product in the sample is indicative of contamination of the test sample by *Alicyclobacillus* or *Geobacillus* or both.

10. The method of claim 9 wherein the forward primer has a sequence selected from the group consisting of SEQ ID NO 38, SEQ ID NO 39, SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 42, and SEQ ID NO 43.

11. The method of claim 9 wherein the reverse primer has a sequence selected from the group consisting of SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 52, and SEQ ID NO 53.

12. The method of claim 9 wherein the probe has a sequence selected from the group consisting of SEQ ID NO 44, SEQ ID NO 45, SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO 48, and SEQ ID NO 49.

13. A method for detecting mold or yeast in a test sample, the method comprising

(a) providing a oligonucleotide set comprising:

(i) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 81 through nucleotide position 225 shown in Figure 8;

(ii) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 81 through nucleotide position 225 shown in Figure 8, said second consecutive sequence being downstream from said first consecutive sequence,; and

(ii) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is which is identical to a third consecutive sequence within the sequence of nucleotide position 81 through nucleotide position 225 shown in Figure 8, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences;

(b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and

(c) determining the presence of PCR products of step (b), wherein the presence of a PCR product in the sample is indicative of contamination of the test sample by *mold or yeast* or both.

14. The method of claim 13 wherein the forward primer has a sequence selected from the group consisting of SEQ ID NO 54, and SEQ ID NO 58.

15. The method of claim 13 wherein the reverse primer has a sequence selected from the group consisting of SEQ ID NO 55, and SEQ ID NO 59.

16. The method of claim 13 wherein the probe has a sequence selected from the group consisting of SEQ ID NO 56, SEQ ID NO 57, and SEQ ID NO 60

17. A method for detecting mold or yeast in a test sample, the method comprising

- (a) providing a oligonucleotide set comprising:
 - (i) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 114 through nucleotide position 238 shown in Figure 8;
 - (ii) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 114 through nucleotide position 238 shown in Figure 8, said second consecutive sequence being downstream from said first consecutive sequence,; and
 - (ii) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is which is identical to a third consecutive sequence within the sequence of nucleotide position 114 through nucleotide position 238 shown in Figure 8, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences;
- (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and
- (c) determining the presence of PCR products of step (b), wherein the presence of a PCR product in the sample is indicative of contamination of the test sample by *mold or yeast* or both.

18. The method of claim 17 wherein the forward primer has a sequence of SEQ ID NO 61.

19. The method of claim 17 wherein the reverse primer has a sequence of SEQ ID NO 62.

20. The method of claim 17 wherein the probe has a sequence of SEQ ID NO 63.

21. The method of claim 1 wherein the primers

- i.) do not contain runs of more than 5 of the same nucleotide base,
- ii) do not contain internal palindromic sequences,
- iii) do not hybridize to one another under stringent conditions, and
- iv) have 40 to 60 percent G+C content, and

wherein said PCR amplification provides a PCR product that is from 50 to 613 nucleotides in length

22. The method of claim 1, wherein the PCR is quantitative PCR.
23. The method of claim 1, wherein the PCR is real-time PCR.
24. A method of detecting the presence of acidic bacteria in a test sample using real time monitoring of a polymerase chain reaction amplification of a target nucleic acid sequence found in the acidic bacteria, said method comprising the steps of
 - (a) adding to the test sample an effective amount of a forward nucleic acid primer and reverse nucleic acid primer and a nucleic acid probe, wherein the forward primer is selected from the group consisting of SEQ ID NO 1, SEQ ID NO 5, SEQ ID NO 9, SEQ ID NO 13, SEQ ID NO 17, and SEQ ID NO 20, and wherein the reverse primer is selected from the group consisting of SEQ ID NO 4, SEQ ID NO 8, SEQ ID NO 12, SEQ ID NO 16, SEQ ID NO 19, and SEQ ID NO 23, and wherein the probe is selected from the group consisting of SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 18, SEQ ID NO 21, and SEQ ID NO 22 and wherein the probe hybridizes to an amplified copy of the target nucleic acid sequence, and wherein the probe is labeled with a marker which emits a signal upon the hybridization of the probe to the target nucleic acid sequence;
 - (b) amplifying the target nucleic acid sequence by polymerase chain reaction;
 - (c) detecting the emitted signal of the sample.
25. A method of detecting the presence of fungi in a test sample using real time monitoring of a polymerase chain reaction amplification of a target nucleic acid sequence found in the acidic bacteria, said method comprising the steps of
 - (a) adding to the test sample an effective amount of a forward nucleic acid primer and reverse nucleic acid primer and a nucleic acid probe, wherein the forward primer is selected from the group consisting of SEQ ID NO 54, and SEQ ID NO 58 and SEQ ID NO 61 , and wherein the reverse primer is selected from the group consisting of SEQ ID NO 55, SEQ ID NO 59, and SEQ ID NO 62, and wherein the probe is selected from the group consisting of SEQ ID NO 56, SEQ ID NO 57, SEQ ID NO 60, and SEQ ID NO 63 and wherein the probe

hybridizes to an amplified copy of the target nucleic acid sequence, and wherein the probe is labeled with a marker which emits a signal upon the hybridization of the probe to the target nucleic acid sequence;

- (b) amplifying the target nucleic acid sequence by polymerase chain reaction;
- (c) detecting the emitted signal of the sample.

26. A kit for detecting *Alicyclobacillus* and *Geobacillus* in a test sample, comprising a set of oligonucleotides comprising:

- (a) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 1327 through nucleotide position 1460 shown in Figure 1;

- (b) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 1327 through nucleotide position 1460 shown in Figure 1, said second consecutive sequence being downstream from said first consecutive sequence; and

- (c) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is identical to a third consecutive sequence within the sequence of nucleotide position 1327 through nucleotide position 1460 shown in Figure 1, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences.

27. A kit for detecting *Alicyclobacillus* and *Geobacillus* in a test sample, comprising a set of oligonucleotides comprising:

- (a) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 334 through nucleotide position 485 shown in Figure 5;

- (b) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 334 through nucleotide position 485 shown in Figure 5, said second consecutive sequence being downstream from said first consecutive sequence; and

(c) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is identical to a third consecutive sequence within the sequence of nucleotide position 334 through nucleotide position 485 shown in Figure 5, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences.

27. A kit for detecting *Alicyclobacillus* and *Geobacillus* in a test sample, comprising a set of oligonucleotides comprising:

(a) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 752 through nucleotide position 813 shown in Figure 5;

(b) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 752 through nucleotide position 813 shown in Figure 5, said second consecutive sequence being downstream from said first consecutive sequence; and

(c) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is identical to a third consecutive sequence within the sequence of nucleotide position 752 through nucleotide position 813 shown in Figure 5, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences.

28. A kit for detecting *yeast or mold* in a test sample, comprising a set of oligonucleotides comprising:

(a) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 81 through nucleotide position 225 shown in Figure 8;

(b) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 81 through nucleotide position 225 shown in Figure 8, said second consecutive sequence being downstream from said first consecutive sequence; and

(c) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is which is identical to a third consecutive sequence within the sequence of nucleotide position 81 through nucleotide position 225 shown in Figure 8, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences.

29. A kit for detecting *yeast or mold* in a test sample, comprising a set of oligonucleotides comprising:

(a) a forward primer of from 15 to 35 nucleotides in length, said forward primer comprising a sequence which is identical to a first consecutive sequence within the sequence of nucleotide position 114 through nucleotide position 238 shown in Figure 8;

(b) a reverse primer of from 15 to 35 nucleotides in length, said reverse primer comprising a sequence which is complementary to the inverse complement of a second consecutive sequence within the sequence of nucleotide position 114 through nucleotide position 238 shown in Figure 8, said second consecutive sequence being downstream from said first consecutive sequence; and

(c) a probe of from at least 15 nucleotides in length, said probe comprising a sequence which is which is identical to a third consecutive sequence within the sequence of nucleotide position 114 through nucleotide position 238 shown in Figure 8, or the complement thereof, and falls between or overlaps with the first and second consecutive sequences.